A Note on the Detection of Leucoanthocyanins in Defatted Soybean Flakes[1]

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During studies on flavor components in soybean flakes we observed evidence of leucoanthocyanins in soybeans. Ethanolic extracts of hydrochloric acid-treated (12N, 5 min. room temperature), defatted, dehulled soybean flakes were an intense red. Absorbance at 550 mμ of such extracts was higher than for similar extracts from corn, barley, wheat, or rye (ten, five, four, and three times higher, respectively). The color extracted from soybeans intensified with increased acidity of the extract. However, color stability seemed to decrease with increased acidity. Attempts to obtain a fraction rich in anthocyanidins from a 1% HCl in 95% ethanol extract of defatted flakes led to a brown product, despite all precautions to prevent air oxidation. Chrysanthenin has been found in the soybean hull (1), and a leucoanthocyanin occurs in leaves of the soybean plant. Such constituents in the

[1]Presented at the 150th meeting of the American Chemical Society, Atlantic City, N. J., September 1965. Contribution from the Northern Regional Research Laboratory, Peoria, Illinois 61604. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

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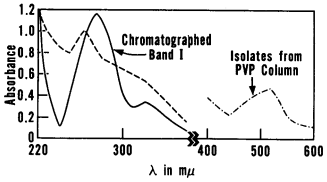

Fig. 1. Ultraviolet and visible spectra of leucoanthocyanin from soybean defatted meal. The isolates were obtained from a PVP column. Band I was isolated by paper chromatography. For details see text.

bean itself have not been recorded. A fraction, which behaved like a leucoanthocyanin, was isolated from extracts of soybean flakes by chromatography on a polyvinylpyrrolidone (PVP) column. This note reports the isolation of such a fraction.

One kilogram of defatted, dehulled soybean flakes of Hawkeye variety was extracted with 1% HCl in 95% ethanol at room temperature for 1 hr. with a flake-to-solvent ratio of 1:10 (w./v.). The mixture was centrifuged to collect the clear supernatant. On evaporation under reduced pressure to remove alcohol, the supernatant yielded a syrupy solid which was then dissolved in a minimum amount of water. This water solution (150 ml.) was put on a PVP column (2 by 40 cm.) prepared as described by Loomis and Battaile (2). The column was washed with water until the absorbance at 280 mμ was 0.1 or lower. Leucoanthocyanins were eluted with 500 ml. 1% HCl in methanol. The methanol was removed under reduced pressure and the lyophilized isolate yielded less than 1 g.

After treatment with concentrated HCl, the isolate yielded a spectrum which peaked at 260, 320, and 520 mμ (Fig. 1). When chromatographed on Whatman No. 1 paper with Forestal solvent (HCl: acetic acid:water, 30:10:3), the sample showed streaks in the anthocyanidin region. After the sample was stored in solution (pH 3 to 4) overnight, the streaks decreased and a spot with R_f value of 0.90 increased. The stored sample was separated into three bands by Whatman No. 3 paper chromatography (R_f 0.90, 0.73, 0.52 in Forestal solvent). Bands 1 (R_f 0.90) and 2 (R_f 0.73) responded to sprayings with diazotized amine reagent, $FeCl_3$ and $Fe_3K(CN)_6$, but weakly to vanillin and *p*-toluene sulfonic acid. Under ultraviolet light band 1 was nonfluorescent, band 2 showed a bluish fluorescence, and band 3 appeared bluish white. Band 1, eluted off the paper with 1% HCl in methanol, absorbed at 275 mμ (Fig. 1) and had a shoulder at 320 mμ. Band 2 absorbed strongly at 260 mμ and was not studied further. The spectrum of band 1 was believed to be that of an anthocyanidin derivative formed during storage in acid solution. This derivative was cochromatographed and its spectrum was compared with similar derivatives from authentic anthocyanidin chlorides. Pelargonidin, cyanidin, and delphinidin chlorides, which gave R_f values of 0.71, 0.53, and 0.32 respectively on paper chromatography (Forestal solvent), were stored in acid solution at room temperature for 48 hr. After 24 hr., peaks in the 500-mμ region decreased about 17% and more absorption in the 400-mμ region developed. After 48 hr., all samples peaked at 275 mμ and the red color diminished. On paper chromatography all derivatives migrated with an R_f of 0.90. The isolate from soybeans was believed to be leucoanthocyanins which did not yield stable anthocyanidins but further decomposed into derivatives.

The conventional procedure (3) to convert leucoanthocyanins to anthocyanidins was unsuitable with extracts from soybean flakes. Perhaps other constituents in soybeans interfered and decreased the stability of the flavylium salts formed. Under the conditions reported here an anthocyanidin-like derivative that absorbed at 275 mμ was isolated.

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[Received February 19, 1969. Accepted March 24, 1969]